

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number
WO 00/73344 A2

(51) International Patent Classification⁷: **C07K 16/00**

(21) International Application Number: **PCT/IT00/00218**

(22) International Filing Date: **26 May 2000 (26.05.2000)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
RM99A000333 26 May 1999 (26.05.1999) IT

(71) Applicant (for all designated States except US): **SIRS SOCIETA' ITALIANA PER LA RICERCA SCIENTIFICA SRL [IT/IT]; Via Giovanni Paisiello, 47/c, I-00198 Roma (IT).**

(72) Inventor; and

(75) Inventor/Applicant (for US only): **NOVAK, Michal, M. [SK/SK]; Svabinskeho Str. n. 1, 851 01 Bratislava (SK).**

(74) Agents: **BANCHETTI, Marina et al.; Ing. Barzanò & Zanardo Roma S.p.A., Via Piemonte, 26, I-00187 Roma (IT).**

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **MONOCLONAL ANTIBODIES, SYNTHETIC AND BIOTECHNOLOGICAL DERIVATIVES THEREOF ACTING AS NGF-ANTAGONIST MOLECULES**

(57) Abstract: Monoclonal antibodies, synthetic and biotechnological derivatives thereof (ScFv or others) are able to recognise the NGF high affinity receptor, TrkA, and act as NGF-antagonist molecules. Pharmacological compositions for therapy, gene therapy, diagnostics of neurological pathologies are also described. Transgenic animal models to study such pathologies are also described.

WO 00/73344 A2

MONOCLONAL ANTIBODIES, SYNTHETIC AND BIOTECHNOLOGICAL DERIVATIVES THEREOF ACTING AS NGF-ANTAGONIST MOLECULES

5 The present invention relates to monoclonal antibodies, to synthetic and biotechnological derivatives thereof, acting as NGF-antagonist molecules.

More particularly the invention relates to a monoclonal antibody, to synthetic and recombinant derivatives thereof able to recognise and bind the high affinity tyrosine kinase receptor of NGF (Nerve Growth
10 Factor), named as TrkA, and act as antagonist for the binding of NGF to TrkA. The invention also concerns diagnostic and therapeutic uses of such molecules, and related compositions.

Neurotrophins are a family of peptide growth factors (Barde, 1994), structurally related to the first member of the family, NGF (Nerve Growth Factor, Levi-Montalcini, 1987). Neurotrophins modulate neuronal
15 differentiation and survival, as well the synaptic transmission, both of peripheral neurons and of the central nervous system. Furthermore NGF acts on various non neuronal tissues and cells, as immune system cells.

NGF acts through two membrane receptors present in the target cells, the low affinity p75 receptor, and the 140 kDa high affinity
20 transmembrane glycoprotein, TrkA (Kaplan et al., 1991, Klein et al., 1991) having a tyrosine kinase activity. TrkA is expressed in neural-crest neurons, in sympathetic neurons as well as in cholinergic neurons of the basal fore-brain and corpus striatum, where it represents the crucial
25 mediator of NGF activities (Holtzman et al., 1992; Verge et al., 1992). TrkA is also expressed in some non neuronal tissues and cells, including B lymphocytes (Torcia et al., 1996).

Prior art suggests the potential use of NGF for the treatment of various neurodegenerative pathologies, including Alzheimer's disease
30 (Lindsay et al., 1994; Ebendal et al., 1991), and other pathologies, as diabetes mellitus and leprosy (Anand et al., 1996). However initial clinical

tests were discouraging, complicated by delivery difficulties, by the pharmacokinetics in the central nervous system, and by NGF negative agonist properties towards other peripheral targets, out of the central nervous system, which lead to excessive and undesired stimuli.

5 Therefore there is the need to develop antagonist molecules selective for the interaction NGF-TrkA receptor and pharmacologically active derivatives thereof, which are easily delivered.

10 Furthermore, the NGF over-production in various inflammatory conditions was related to the increase of pain sensitivity of the primary afferent nociceptors, thus contributing to the occurrence of a chronic pain condition. The population of sensorial neurons that are sensitive to tissue damages (nociceptors) is particularly NGF-dependent. In addition, considering the disadvantages and limitations of the two existing analgesic drug classes (non steroidal anti-inflammatory drugs and opiates), the
15 provision of a new different target, as NGF, represents a progress in the art (Snider and McMahon, 1998). And further, as suggested by Levine (Levine, 1998) NGF provides a potential target for the design of new therapies of the pain, specially those resulting from inflammatory or neuropathic conditions, for which conventional drugs are less effective.
20 Finally new studies showed a direct relation between pain and TrkA system demonstrating, in four unrelated cases of type 4 pain chronic insensitivity, with anhidrosis, the presence of mutations of the TrkA gene and consequently the absence of functional NGF receptors (Indo et al., 1996; Wood, 1996).

25 Accordingly the NGF-TrkA system provides a potential target to design pain therapies, i.e. treatments able to antagonise the pain neuropathic syndrome by means of TrkA-effective antagonists (Levine, 1998; Snider and McMahon, 1998).

30 The aberrant expression of the TrkA receptor mRNA was related also to neoplastic pathologies. The prognosis of TrkA expressing tumours, the "imaging" diagnostic and the therapy as well represent an application area of antibodies having an high affinity for TrkA. As a matter of fact, in these tumours TrkA binding agents represent useful tools of the

clinical diagnosis, prognosis and therapeutic treatments (Kramer et al., 1997).

Recombinant antibodies (Vaughan et al., 1998) represent starting reagents of choice for the development of small molecule mimicking their activity (Le Sauteur et al., 1995).

A TrkA-agonist monoclonal antibody was described by Le Sauteur et al., 1996 and in PCT application No. WO97/21732. The agonist activity of the only disclosed antibody (5C3) makes it not suitable for the aims of the present invention and in all the situations wherein the hyper-activation of the TrkA receptor must be avoided. PCT application No. WO97/21732 discloses the use as "imaging" diagnostic of the agonist antibody 5C3. However this antibody, due to its agonist activity, can not be used for the above application, unless the receptor activation is not hampered.

Therefore it is clear the need for the development of new molecules suitable to interfere with the binding of NGF to TrkA, to provide new therapeutic activities and, particularly, to provide a TrkA antibody acting as antagonist and then ideal for blocking the receptor activation by endogenous ligand (NGF), and having no activation activity of the receptor. Furthermore the antibody could be advantageously used for the development of reagents, i.e. synthetic and recombinant fragments blocking the NGF-TrkA interaction.

The author of the present invention isolated various monoclonal antibodies able to interact with the NGF-receptor, named TrkA. Among these an antibody, named MNAC13, acts as a strong antagonist of TrkA, by inhibiting the binding of NGF to TrkA. This antibody represents a very effective tool in preventing the functional activation of TrkA by NGF in a variety of biological systems.

The antibody was derived by congenic immunization of Balb/C mice, with a human native TrkA receptor expressed on Balb/C 3T3 cells. The screening was based on the ability of the antibodies to inhibit the binding of NGF to TrkA-expressing cells. This led to the isolation of antibodies able to bind TrkA at its NGF-binding domain, thus preventing

the binding of NGF. The antibody MNAC13 is very effective in preventing the functional activation of TrkA by NGF in different biological systems. The author of the present invention cloned also the genes encoding the variable regions of the MNAC13 antibody and, by means of recombinant DNA techniques, assembled such regions in a functional polypeptide of reduced size (single chain Fv fragment, scFvMNAC13), confirming that it retains the properties of the parental antibody.

An agonist antibody means an antibody able to activate the receptor antigen in the absence of the native ligand of the receptor itself.

An antagonist antibody means an antibody directed against the active site of the antigen receptor and able to inhibit the activity of the natural ligand being in competition with the latter for binding to the receptor itself.

Synthetic and biotechnological derivatives of an antibody mean any engineered fragment, synthesised by chemical or recombinant techniques, which retain the functional properties of the antibody.

It is an object of the present invention a monoclonal antibody, synthetic and biotechnological derivatives thereof, able to recognise and bind the high affinity tyrosine kinase receptor of NGF (Nerve Growth Factor), named as TrkA, and act as antagonist for the binding of NGF to TrkA.

According to a preferred embodiment, the antibody of the invention has the light chain variable region essentially consisting of the sequence from aa. 23 to aa. 134 of SEQ ID No. 2.

According to a further preferred embodiment, the antibody of the invention has the heavy chain variable region essentially consisting of the sequence from aa. 152 to aa. 276 of SEQ ID No. 2

According to a further preferred embodiment the biotechnological derivative of the invention is a ScFv fragment comprising:

a) the light chain variable region of the antibody of the invention or functional derivatives thereof, and

b) the heavy chain variable region of the antibody of the invention or functional derivatives thereof.

Preferably the ScFv fragment comprises a linker sequence between light chain and heavy chain variable regions. More preferably the ScFv fragment has essentially the sequence of SEQ ID No. 2.

5 It is a further object of the invention a synthetic or biotechnological derivative of the monoclonal antibody which comprises at least one region determining the complementarity of the antibody (CDR) and which is able to act as antagonist for the binding of NGF to TrkA. Preferably the region is within the variable region of the heavy chain, more preferably the region is comprised in the sequence from aa. 152 to aa. 276
10 of SEQ ID. No. 2.

It is within the scope of the present invention a nucleic acid encoding the antibody or derivatives thereof of the invention. Preferably the nucleic acid encodes the ScFv fragment of SEQ ID No. 2, more preferably the nucleic acid has the sequence of SEQ ID No. 1.

15 The nucleic acids of the invention can be advantageously used as transgenes to obtain non human transgenic animals, preferably mice, wherein the antibody is expressed in an inducible way, or under the control of promoters which determine the expression in the adult animal. These animals can be advantageously used to study and test drugs for
20 human pathologies wherein the NGF/TrkA interaction is inhibited and, particularly, neurodegenerative pathologies. Transgenic non human animals can be obtained with standard techniques, i.e. as described in Allen et al., 1987.

Transgenic models (Smeyne et al., 1994) based on the
25 repression of the TrkA gene show a lethal phenotype within 1-2 weeks from birth, and are then unsuitable to study TrkA in the adult and aged nervous system. Antibody expressing transgenic animals are disclosed by Piccioli et al., 1991, 1995.

30 It is within the scope of the present invention a phage or a prokaryotic vector comprising and able to express correctly and effectively the nucleic acid of the invention.

It is within the scope of the present invention a recombinant eukaryotic vector comprising and able to express correctly and effectively

the nucleic acid of the invention, as well as a pharmacological composition comprising the recombinant vector for gene therapy of neurological pathologies. Pathologies comprise, but are not limited to, the following group: chronic pain, acute pain, neuromas, TrkA expressing neoplastic tumours.

It is within the scope of the present invention a pharmacological composition comprising an effective amount of the monoclonal antibody of the invention, or of synthetic and biotechnological derivatives thereof, able to recognise and bind to the high affinity tyrosine kinase receptor of NGF (Nerve Growth Factor), named as TrkA, and act as antagonist for the binding of NGF to TrkA, and a pharmaceutically acceptable carrier. The composition of the invention can be advantageously used for the treatment of neurological pathologies comprised, but not limited to, the following group: chronic pain, acute pain, neuromas, TrkA expressing neoplastic tumours.

In consideration of the fact that NGF may have some undesired collateral effects in therapy, the invention relates also to a pharmaceutical composition comprising pharmaceutically active amount of NGF and of the antibody or derivatives thereof according to the invention. Such composition should be able to inhibit at peripheral level the undesired effects of NGF.

It is a further object of the present invention engineered cells able to express the antibody of the invention or biotechnological and synthetic derivatives thereof, as well a pharmacological composition comprising said cells for gene therapy of neurological pathologies comprised but not limited to the following group: chronic pain, acute pain, neuromas, TrkA expressing neoplastic tumours.

In view of the specificity of the antibody of the invention and in the absence of undesired inducing effects, it can be advantageously used in a composition for in vivo "imaging" diagnostics.

The present invention will be described with reference to exemplifying, but not limiting, embodiments thereof. Reference will be done to the following Figures.

Figure 1 Inhibition of binding of 125 I-NGF to TrkA+ Balb/C 3T3 cells. Hybridoma supernatants were pre-incubated with TrkA+ Balb/C 3T3 cells, prior to the addition of 125 I-NGF. The histogram reports the inhibition of specific binding NGF-cell by different antibodies. The specific binding was evaluated by subtracting from the total binding that obtained in the presence of an excess of unlabelled NGF. The values reported are the mean of triplicates.

Figure 2. MNAC13 recognises the extracellular domain of the TrkA receptor. Soluble TrkA and TrkB receptors, engineered as immunoadhesins, were used as solid phase antigens for an ELISA assay and incubated with 2 or 20 ng/ml of purified MNAC13 antibody.

Figure 3 MNAC13 recognises the TrkA receptor on living cells. Balb/C 3T3 or TrkA+ Balb/C 3T3 cells were incubated with MNAC13 antibody and subjected to FACS analysis.

Figure 4 MNAC13 labels the TrkA receptors on rat basal forebrain neurons. Coronal sections of P10 rat basal forebrain were incubated in the presence (A) or in the absence (B) of MNAC13 antibody. Scale bar: 98 μ m.

Figure 5 MNAC13 inhibits the NGF induced differentiation of rat PC12 cells. PC12 cells were transferred to serum-free medium and incubated in the absence (A) or in the presence (B, C and D) of 20 ng/ml NGF for about 4 days. The MNAC13 antibody (4 μ g/ml) inhibits completely NGF-induced survival and differentiation, while the control antibody 9E10 does not (D).

Figure 6 Implant of MNAC13 secreting cells in the rat brain significantly reduces the number of cholinergic basal forebrain neurons. The cholinergic phenotype of P9 rat basal forebrain neurons was determined by immunoreactivity with the choline acetyltransferase (ChAT), further to intraventricular implant of MNAC13 ibridomas (B) of control myeloma (A) cells at P2 day. Note the marked reduction of the number of ChAT positive neurons in MNAC13 implanted rats (B). Scale bar. 65 μ m.

Figure 7 Recombinant forms of MNAC13 mAB bind TrkA. Phages with scFvMNAC13 (A), soluble scFvMNAC13 (B) and the parental monoclonal

antibody MNAC13 (C) were used in an ELISA assay using TrkA immuno-adhesin as solid phase antigen in the presence of increasing concentrations of competing soluble TrkA-immuno-adhesin. ■: ten-fold dilution of the antibody with respect to ▲.

- 5 Figure 8. Inhibition of NGF-induced neurite growth of PC12 cells by the recombinant antibody ScFvMNAC13. Periplasmic fraction containing the recombinant ScFvMNAC13 (B) or ScFv control fragment (antifox ScFv) were added, together with 10 ng/ml of NGF to PC12 cells induced for 7 days with 50 ng/ml of NGF and plated again at the beginning of the assay.
- 10 The recombinant ScFvMNAC13 antibody in B inhibits the re-growth of neurites in replated NGF induced PC12 cells, mediated by the activation of TrkA by NGF.

METHODS

Immunization protocol

- 15 Balb/C 3T3 transfected cells, expressing 10^6 human TrkA molecules per cell were used in a congenic immunization protocol. Three groups of female Balb/C mice were immunized with 10^5 , 5×10^5 and 10^6 living cells per mouse, respectively. After five injections at two week intervals pre-fusion sera were tested for their ability to inhibit the binding of
- 20 NGF to the TrkA receptor on TrkA+ Balb/C 3T3 cells. The greatest inhibition of NGF binding was found in the sera from mice injected with 5×10^5 cells (binding inhibition at a 1/100 dilution).

Hybridoma production

- 25 Three days after a boost injection of TrkA+ Balb/C 3T3 cells, the mice were sacrificed, the spleens removed and splenocytes were fused to NSO myeloma (10:1 ratio) with polyethylene glycol (PEG 1500), as described (Novak et al., 1991). The hybridoma growth and selection were performed according to standard methods (Galfre and Milstein, 1981).

- 30 Inhibition of 125 I binding to TrkA+ Balb/C 3T3 Cells

2,5 S NGF was purified from mouse submandibular glands and was iodinated to a specific activity of 10^5 cpm/ng as described (Cattaneo et al. 1983). 5×10^4 TrkA+ Balb/C 3T3 cells were plated in each well of 96

well microplates in a volume of 50 μ l of culture medium (DMEM with FCS 10%) . Aliquots of 50 μ l of hybridoma supernatant were incubated for 1 hour with cells, followed by the addition of the 125 I-NGF solution (5×10^4 cpm/well). The plates were processed as described (Cattaneo et al., 1988). Non specific binding was determined in parallel wells, in the presence of an excess (5 μ g/ml) of unlabelled NGF. In parallel wells the binding was carried out in the presence of a non relevant hybridoma supernatant (Rab50) or of neutralizing anti-NGF (mAB α D11, Cattaneo et al., 1988).

10 ELISA

Soluble TrkA and TrkB receptors were engineered as immunoadhesins (Chamow and Ashkenazi, 1996) by linking the extracellular domain of the human TrkA receptor to the Fc portion of IgG2, constituted of a sequence of 35 amino acids, followed by CH2 and CH3 domains. The DNA sequences coding for the TrkA and TrkB immunoadhesins (TrkA-IgG and TrkB-IgG) were cloned into baculovirus (Autographa Californica nuclear polyhedrosis virus, AcNPV) for expression in insect cells (Baculogold transfection kit, Pharmingen Ing.) and the proteins were purified by Protein A-Sepharose chromatography from serum free culture medium of High Five insect cells. For ELISA assay TrkA-IgG and TrkB-IgG were incubated at 2 μ g/ml and then with 2 or 20 ng/ml of MNAC13 and anti mouse IgG, previously pre-absorbed on camel immunoglobulins.

Immunofluorescence Analysis

25 MNAC13 monoclonal antibody was purified from serum free hybridoma supernatants by Protein A-Sepharose chromatography. 5×10^4 TrkA+ 3T3 Balb/C cells were incubated with MNAC13 purified antibody and analysed on an activated cell sorter (FACS). For immunofluorescence adherent cells were fixed with 3,7 % paraformaldehyde in PBS, incubated with purified MNAC13, followed by FITC labelled anti mouse IgG and analysed by confocal microscopy (Olympus).

NGF biological assay with PC12 cells

Rat PC12 cells (Greene and Tischler, 1976) were cultured in RPMI with 10% heat inactivated horse serum and 5% FCS. For the bioassay the cells were transferred in serum free medium and incubated with 20 ng/ml NGF for 4 to 6 days, in the presence or in the absence of MNAC13 antibodies or of its single chain recombinant Fv version (scFvMNAC13). Alternatively the cells were incubate with 50 ng/ml of NGF for one week and then were mechanically removed from neurites for replating in the presence of 10 ng/ml of NGF and the addition of the appropriate antibody. The neurite growth was scored 24-48 hours later.

Intraventricular hybridoma injections and immunochemistry

Intraventricular hybridoma injection and analysis of the cholinergic phenotype of basal forebrain neurons were performed essentially as described (Molnar et al., 1997 and 1998). Briefly MNAC13 hybridoma cells and control myeloma cells (P3X63Ag8) cells were resuspended in Hank (HBBS) solution at 2×10^5 cells/ μ l and injected into the right lateral ventricle of Wistar rats as described (Molnar et al., 1998). The injection was carried out at postnatal day 2 (P2) and the animals were sacrificed for analysis at P8 day. After perfusion under anaesthesia the brains were processed for aChAT immunohistochemistry as described (Molnar et al. 1997 and 1998). The level of MNAC13 antibodies in the cerebrospinal fluid (CSF) was determined by ELISA assay using soluble TrkA receptors as solid phase antigens.

For immunochemistry with MNAC13 the animlas were anaesthetized with ether a perfused with PB (0,1 M, pH 7,4) followed by 4 % paraformaldehyde/PB at 4°C for 2 hours. After dissection the brains were fixed in 4 % paraformaldehyde/PB at 4°C for 2 hours, transferred in 25 % sucrose/PBS, then frozen in isopentane at -20°C and sectioned with a cryostat. The coronal sections containing the basal forebrain were collected on gelatinized slides and stored at -20°C until processing. After blocking non specific binding in a solution of 10 % FCS/5 % BSA in Tris HCl 0,1M pH 7,4, 0,05% Triton-100 the sections were incubated overnight at 4°C with anti-TrkA (6 μ g/ml) in 10% FCS/2% BSA in Tris HCl 0,1M, pH

7,4, 0,05% Triton X-100. The next days the sections were incubated with anti-mouse IgG biotinylated for 2 hours at room temperature and for 1 hour in ABC kit (Vector). The reaction was developed in 3,3'-diaminobenzidine HCl. After dehydration the sections were mounted in DPX.

Cloning of the variable regions of mAb MNAC13.

The cloning of the variable regions of the mAb MNAC13 was carried out from hybridoma mRNA by variable region PCR. Variable region PCR was carried out with a set of oligonucleotide primers for mouse immunoglobulins (Krebber et al., 1997). The amplified VH and VK variable regions were assembled in a scFv format by PCR and cloned into pDNA vector (Bradbury et al., 1996). After finger printing analysis with BstNI restriction endonuclease, which confirmed a limited diversity of the resulting scFVs, phage particles displaying scFv fragments were subjected to ELISA using TrkA-IgG as solid phase antigen. The assay was developed with secondary HRP-coupled anti M13 antibodies. Positively identified phages were further assayed and finally used to produce soluble scFv fragments in E. coli. Bacterial supernatants were assayed by ELISA against TrkA-IgG, using a monoclonal antibody against SV5 tag (Hanke et al., 1992) present in the scFv fragment, followed by HRP-conjugated anti mouse IgG.

RESULTS

Production and characterization of a monoclonal antibody that inhibits the binding of NGF to TrkA

In order to produce antibodies able to interfere with the neurotrophin binding activity of the TrkA receptor was used a congenic immunization protocol. Balb/C-3T3 cells expressing the human TrkA receptor produced by transfection of the human trk proto-oncogene were used for the immunization di Balb/C mouse. The number of cells was found to be critical for the induction of serum antibodies neutralizing NGF binding to target cells.

The hybridoma supernatants were assayed for their ability in inhibiting the binding of ¹²⁵I to 3T3-TrkA+ cells. Out of 1266 wells in which

hybridoma growth was occurring only 4 showed a NGF neutralizing activity. The corresponding cells were subcloned obtaining MNAC13, C30, C191 and C232 clones. The ability of the antibodies produced by these clones to inhibit the binding of NGF to 3T3-TrkA+ cells is showed in Figure 1. These anti-TrkA antibodies inhibit the binding of NGF as efficiently as the neutralizing anti-NGF α D11 antibody (Figure 1). While the latter binds the active site of NGF the former binds the TrkA receptor, most likely at or near to the NGF recognising site. The IgG MNAC13 antibody was selected for further studies.

The inhibition of NGF binding is obtained by a direct interaction of the antibodies with the extracellular portion of the TrkA receptor, as demonstrated by a variety of binding studies on a soluble form of human TrkA receptor, engineered as immunoadhesin (Chamow and Ashkenazi, 1996) in which the extracellular portion of the receptor is fused to the FC domains of the camel immunoglobulins (see Methods). Figure 2 shows that MNAC13 antibody binds the TrkA immunoadhesin in an ELISA assay, while it does not react with TrkB immunoadhesin. This confirms that the MNAC13 antibody binds specifically to the extracellular portion of TrkA.

Figure 3 shows the result of a FACS analysis on 3T3 TrkA+ cells demonstrating that the MNAC13 antibody interacts with the human receptor expressed on the membrane of living cells. An immunofluorescence analysis confirms this result.

The species specificity of MNAC13 antibodies was tested on the base of its ability to recognise TrkA receptors on rat neurons. Sections of rat brains were taken from the basal forebrain region (Figure 4) which is rich in TrkA positive neurons. The intense staining obtained in the basal forebrain with MNAC13 antibody (Figure 4A) shows that this antibody, obtained against the human TrkA receptor, recognises also its rat counterpart. The antibody does not stain brain regions such as the medial habenular nuclei, known to be lacking of positive TrkA neurons (Holtzmann et al., 1995).

Functional block of TrkA-mediated biological actions by MNAC13 antibody

The ability of MNAC13 antibody to inhibit in vivo the biological activation of the TrkA receptor by the NGF ligand was therefore studied in PC12 cells in vitro (Figure 5) as well as in vivo (Figure 6).

5 The NGF-induced differentiation of PC12 cells (Figure 5B) is completely inhibited by incubation of the cultures with MNAC13 antibody (Figure 5C), as compared to incubation with a non relevant antibody (Figure 5D).

10 The cholinergic neurons of the basal forebrain are a well known target for NGF action in the central nervous system (Korsching, 1986; Holtzman et al., 1992). The hybridoma cells secreting the MNAC13 antibody were implanted in the lateral ventricle of neonatal rats two days after birth and the cholinergic phenotype of the neurons was studied a week (P9) later by immunohistochemistry with antibodies against

15 cholineacetyltransferase (ChAT). This experimental approach has been used recently to study the effects of implanted cells secreting the anti-NGF monoclonal antibody α D11 (Berardi et al., 1994; Molnar et al., 1997 and 1998). One week after the implant the level of anti-TrkA antibodies found in the cerebrospinal fluid, determined by ELISA, was of 1,4 ng/ μ l. The

20 results in Figure 6 show that the number of ChAT positive cells is dramatically reduced in the brains implanted with the anti-TrkA antibody with respect to the controls (injected with a non relevant myeloma). A quantitative evaluation of the number of the positive ChAT neurons showed that this number is reduced by 70 % in the medial septum and by

25 77% in the diagonal band of rats implanted with MNAC13 antibody, with respect to the controls. An extension of this study to various post-natal ages, comparing the effects obtained in a previous study, with implant of cells secreting an anti-NGF neutralizing antibody (Molnar et al., 1997, 1998) showed that the deprivation effects of the cholinergic system of the

30 basal forebrain obtained with the anti-TrkA MNAC13 antibody are much more severe.

Isolation of a recombinant functional form of mAB MNAC13 with a significantly reduced size

In order to expand the range of application the variable regions of this antibody were cloned and engineered into a recombinant antibody of smaller size.

5 The cloning of the variable regions of monoclonal antibodies from the corresponding hybridoma can be complicated leading to the cloning of artifactual variable regions. Therefore the author used the technique according to Winter et al. (1994). The variable heavy (VH) and light (VL) regions of the MNAC13 antibody were amplified by PCR from a cDNA derived from hybridoma mRNA using mouse IgG specific
10 oligonucleotide primers. The variable regions were assembled into single chain Fv (scFv) by PCR and cloned into pDAN vector to allow the expression of antibody fragments cloned on the surface of filamentous phage. ScFv fragments represents a biotechnological derivative of the original antibody (Bird et al., 1988) and consist of the variable light and
15 heavy regions joined to a linker peptide which links the C terminus of the VL region to the N terminus of the VH region. The nucleotide sequence of ScMNAC13 specific fragment is showed in SEQ ID No. 1. The amino acid sequence is showed in SEQ ID No. 2 in which the VL region is from aa. 23 to aa. 134, the VH region is from aa. 152 to aa. 276.

20 The CDRs (regions determining the antibody complementarity) are three for each chain, and particularly:

VL CDR1 aa. 46-55 of SEQ ID No. 2;
VL CDR2 aa. 71-77 of SEQ ID No. 2;
VL CDR3 aa. 110-1119 of SEQ ID No. 2;
25 VH CDR1 aa. 176-185 of SEQ ID No. 2;
VH CDR2 aa. 200-216 of SEQ ID No. 2;
VH CDR3 aa. 249-262 of SEQ ID No. 2.

The sequence of the light and heavy variable regions of the MNAC13 antibody was compared to that of the antibody described in
30 patent application PCT No. WO97/21732, as showed in Tables 1 and 2.

Table 1 Alignment of the light chain of 5C3 and MNAC13

```

5          38
          DILQTQSPAILSASPGEKVTMTCRASSSVSYMHWYQQK
          |||||
MKYLLPTAAAGLLLLLAASGAHADIVLSQSPAIMSASLGEEITLTCSASSSVSYMHWYQQK
          60
          98
10         PGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQWSSNPLTFG
          ||||| ||||| ||||| ||||| |||||
          SGTSPKLLIYTTSNLASGVPSRFSGSGSGTFYSLTISSEVEAEDAADYYCHQWSSYPWTFG
          120
          105
          AGTKLEI
          |||||
          GGTKLEIKRSGGST
          134

```

Table 2 Alignment of the heavy chain of 5C3 and MNAC13

```

20      VQLQESGTVLARPVGASVKMSCKASGYTFTSYWMHWVKQRPQGGLWIGAIYPG 54
          |||                |||                |||
SSSGTEVKLMESGGGLVQPGGSLKLSA-ASGFTFSTYTMSWARQTPEKRLWVAYISKG 59
25      DSDTSYNQKFKGEAKLTAVTSTSTAYMELSSLTNEDSAVYYCTL---YGNYESYYAMDYW 111
          |||                |||                |||        |||
GGSTYYPDTPVKGRFTISRDNAKNTLYLQMSSSLKSEDTALYYCARGAMYGNDFFP-MDYW 118
30      121
      GQGILSHRLL
          |||
      GQGTSVTVSSASG
          131
35

```

The PCR assembled ScFv fragments were expressed on the filamentous phage, as fusion to the p3 phage protein. The minilibrary of phage particles was screened by an ELISA assay on phages using the TrkA immunoadhesin as solid phase antigen. This led to the isolation of positive phages which express on their surface the ScFv version of the MNAC13 parental antibody (scFvMNAC13). The binding properties of this phage, as well as those of the scFv soluble fragment derived from this phage were characterized by a competition ELISA assay (Figure 7). The TrkA immunoadhesin was coupled in solid phase and incubated with MNAC13 expressing phage particles (Figure 7A), with ScFvMNAC13 soluble antibody (Figure 7B) or with parental MNAC13 antibody (Figure 7C), in the presence of increasing amounts of competing soluble TrkA immunoadhesin. The results confirm the ScFv version of the parental

monoclonal antibody either on phage or secreted by E. coli binds TrkA as efficiently as the parental monoclonal antibody.

5 The biological activity of ScFVMNAC13 was tested on PC12 cells. The cells were incubated for one week with 50 ng/ml of NGF, after which they were replated in the presence of NGF and the antibody ScFVMNAC13 fragment. As shown in Figure 8 ScFVMNAC13 fragment inhibits dramatically the extension of neurites from PC12 cells, confirming that the recombinant single chain Fv of the MNAC13 antibody retains the neutralization properties of the parental antibody. The small size of this
10 single polypeptide antibody expands the range of applications of the antibody of the invention facilitating its delivery and expression within the nervous tissue.

Nociception Test

15 The MNAC13 antibody was used in a nociception test for determination of the pain sensitivity by "hot plate test". The experiment was carried out according to McMahon et al. (1995), using the antibody MNAC13 as immunoadhesin. The antibody was infused subcutaneously into hind paw of an adult rat for a period of three weeks or by an osmotic mini-pump. The nociception sensitivity was evaluated at intervals using hot plate test
20 (Eddy and Leimbach, 1953), which mimics hyperalgesia situations following inflammation or partial damage to the nerve. The nociceptive stimulus induces in such a case a response (paw licking and/or jumping) which presumes an integrated coordination higher than simple reflex. According to the test the animal is put in a pen having a plate heated to
25 the desired temperature as base, usually 56°. The latency of any of two responses (paw licking and jumping) is measured in control animals (treated with non relevant antibody) and in those treated with anti-TrkA antibody. The result of the experiment demonstrated the occurrence of a remarkable hypoalgesia, as pointed out by a significant increase of the
30 latency in the MNAC13 treated group.

BIBLIOGRAPHY

- Allen, N.D., et al. (1987). In Mammalian development: A practical approach, M. Monk, ed. (Washington DC: IRL Press) pp 217-234.
- Anand et al. (1996) *Nature Medicine* 2, 703-707.
- Barde YA (1994) *J. Neurobiol* 25, 1329-1333
- 5 - Berardi N. et al. (1994) *Proc. Natl. Acad. Sci USA* 91:684-688
- Bird RE, et al. (1988) *Science* 242:423-425.
- Bradbury A, Cattaneo A, Hoogenboom H (1996) In: *Manual of the EMBO theoretical and practical Course*, pp. 1-122, Trieste 18-26 November
- 10 - Cattaneo A, et al. (1983) *Eur. J. Biochem* 135:285-290.
- Cattaneo A, Rapposelli B, Calissano P (1988), *J. Neurochem* 50:1003-1010.
- Chamow SM, Ashkenazi A (1996), *Trends in Biotechnol* 14:52-60.
- Ebendal T, et al. (1991) In: *Plasticity and regeneration in the nervous system* (Timiras P., Privat A, eds.), New York: Plenum Press
- 15 - Eddy NB and Leimbach DJ (1953) *J. Phar. Exp. Ther.* 107:385-393.
- Galfrè G, Milstein C (1981) *Methods Enzymol.* 73:3-45.
- Greene LA, Tischler AS (1976) *Proc. Natl. Acad. Sci. Usa* 73:2424-2428.
- 20 - Hanke T, Szawlowski P, Randall RE (1992) *J. Gen. Virol.* 73:653-60.
- Holtzman DM, et al. (1992) *Neurin.* 9:465-478.
- Holtzman DM, et al. (1995) *J. Neuroscience* 15:1567-1576.
- Jones PT, et al. (1986) *Nature* 321:522-525.
- Kaplan DR et al. (1991) *Science* 252:554-558.
- 25 - Klein R, et al. (1991) *Cell* 65:189-197.
- Korsching S (1986) *Trends in Neurosci* 9:570-573.
- Krebber A, et al. (1997) *J. Immunol. Methods* 15:35-55.
- Kramer K, et al. (1997) *Eur. J. Cancer* 33:2090-2091.
- Indo et al. (1996) *Nature Genetics* 13, 485-488.
- 30 - LeSauter L. et al. (1995) *J. Biol. Chem.* 270:6564-6569.
- LeSauter L., et al. (1996) *J. Neurosci.* 16, 1308-1316.
- Levi-Montalcini R (1987) *EMBO J* 6, 1145-1316.
- Levi-Montalcini R, Angeletti PU (1996) *Pharmacol. Rev.* 18:619-628.

- Levine ID (1998) Neuron 20:649-654.
- Lindsay RM et al. (1994) Trends in Neuroscience, 17, 182.
- McMahon et al. (1995) Nature Medicine 1, 774-780.
- Molnar M. et al. (1997) NeuroReport 8:575-579
- 5 - Molnar M. et al. (1998) Eur. J. Neurosci. 10: 3127-3140.
- Novak M. et al. (1991) Proc. Natl. Acad. Sci. USA 88:5837-5841
- Piccioli P. Et al. (1991) Proc. Natl. Acad. Sci. USA 88:5611-5615.
- Piccioli P. Et al. (1995) Neuron 15:373-384.
- Smeyne RJ, et al. (1994) Nature 368:246-249.
- 10 - Sinder WD, McMahon SB (1998) Neuron 20:629-632.
- Torcia M, et al. (1996) Cell 85:345-356.
- Vaughan TJ, Osbourne JK, Tempest PR (1998) Nature Biotech 16, 535-539.
- Verge VMK, et al. (1992) J. Neurosci. 12:4011-4022.
- 15 - Winter G, et al. (1994) Annu. Rev. Immunol. 12:433-455.
- Wood (1996) Nature Genetics 13, 382-383.

CLAIMS

1. Monoclonal antibody, synthetic and biotechnological derivatives thereof, able to recognise and bind the high affinity tyrosine kinase receptor of NGF (Nerve Growth Factor), named TrkA, and act as antagonist for the binding of NGF to TrkA.

2. Monoclonal antibody, synthetic and biotechnological derivatives thereof according to claim 1 wherein the variable region of the light chain has essentially the sequence from aa. 23 to aa. 134 of SEQ ID No. 2.

3. Monoclonal antibody, synthetic and biotechnological derivatives thereof according to claim 1 wherein the variable region of the heavy chain has essentially the sequence from aa. 152 to aa. 276 of SEQ ID No. 2.

4. Monoclonal antibody, synthetic and biotechnological derivatives thereof according to any of previous claims wherein the variable region of the light chain has essentially the sequence from aa. 23 to aa. 134 of SEQ ID No. 2 and the variable region of the heavy chain has essentially the sequence from aa. 152 to aa. 276 of SEQ ID No. 2.

5. A ScFv fragment of the monoclonal antibody according to any of previous claims comprising at least one variable region of the light chain or of the heavy chain of the antibody as described in claim 1.

6. The ScFv fragment according to claim 5 comprising variable region of the light chain and of the heavy chain of the antibody as described in claim 1.

7. The ScFv fragment according to claim 6 comprising a linker sequence between the variable region of the light chain and the variable region of the heavy chain.

8. The ScFv fragment according to claim 7 wherein said ScFv fragment has essentially the sequence of SEQ ID No. 2.

9. Synthetic or biotechnological derivative according to claim 1 comprising at least one region determining the complementarity of the antibody (CDR) and which is able to act as antagonist for the binding of NGF to TrkA.

derivatives thereof according to any of claims 1-10, able to recognise and bind the high affinity tyrosine kinase receptor of NGF (Nerve Growth Factor), called TrkA, and to act as antagonist for the binding of NGF to TrkA and a pharmaceutically acceptable carrier.

5 20. Pharmacological composition according to claim 19 for the treatment of neurological pathologies comprised within but not limited to the following group: chronic pain, acute pain, neuromas, TrkA expressing neoplastic tumours.

10 21. Pharmaceutical composition comprising pharmaceutically active amounts of NGF and of the antibody or derivatives thereof according to any of claims 1-10.

22. Eukariotic engineered cells able to express the antibody or derivatives thereof according to any one of claims 1-10.

15 23. Pharmacological composition comprising cells according to claim 22 and a pharmaceutically acceptable carrier for gene therapy of neurological pathologies comprised within but not limited to the following group: chronic pain, acute pain, neuromas, TrkA expressing neoplastic tumours.

20 24. Composition comprising an effective amount of the monoclonal antibody or synthetic or biotechnological derivatives thereof according to any of claims 1-10, able to recognise and bind the high affinity tyrosine kinase receptor of NGF (Nerve Growth Factor), called TrkA, and to act as antagonist for the binding of NGF to TrkA and a diagnostically acceptable carrier for use in in vivo "imaging" diagnostics.

25

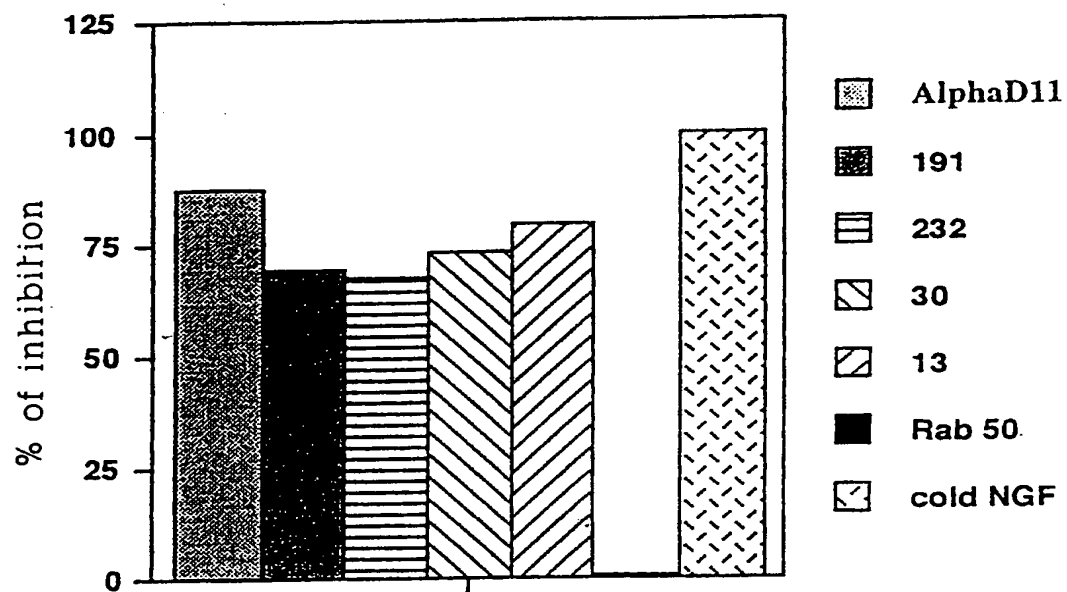


FIG. 1

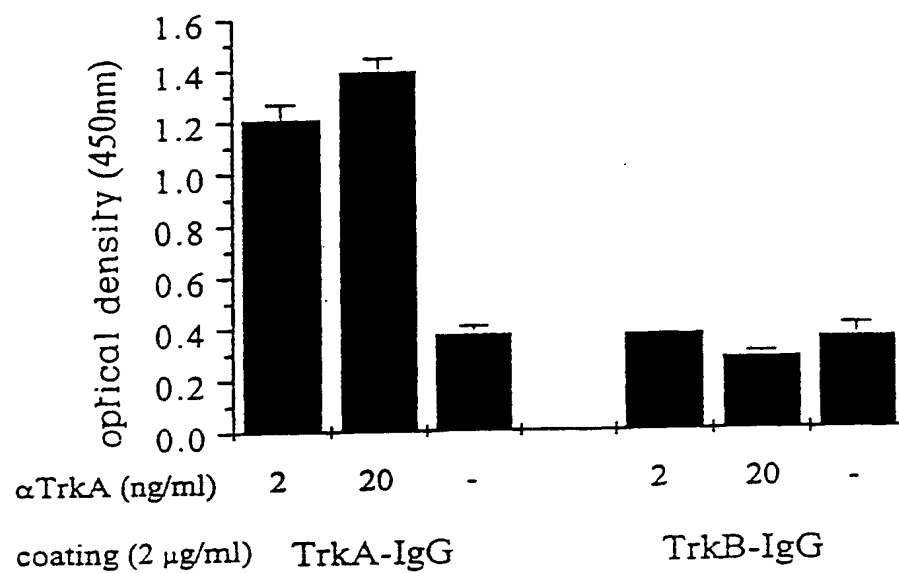


FIG. 2

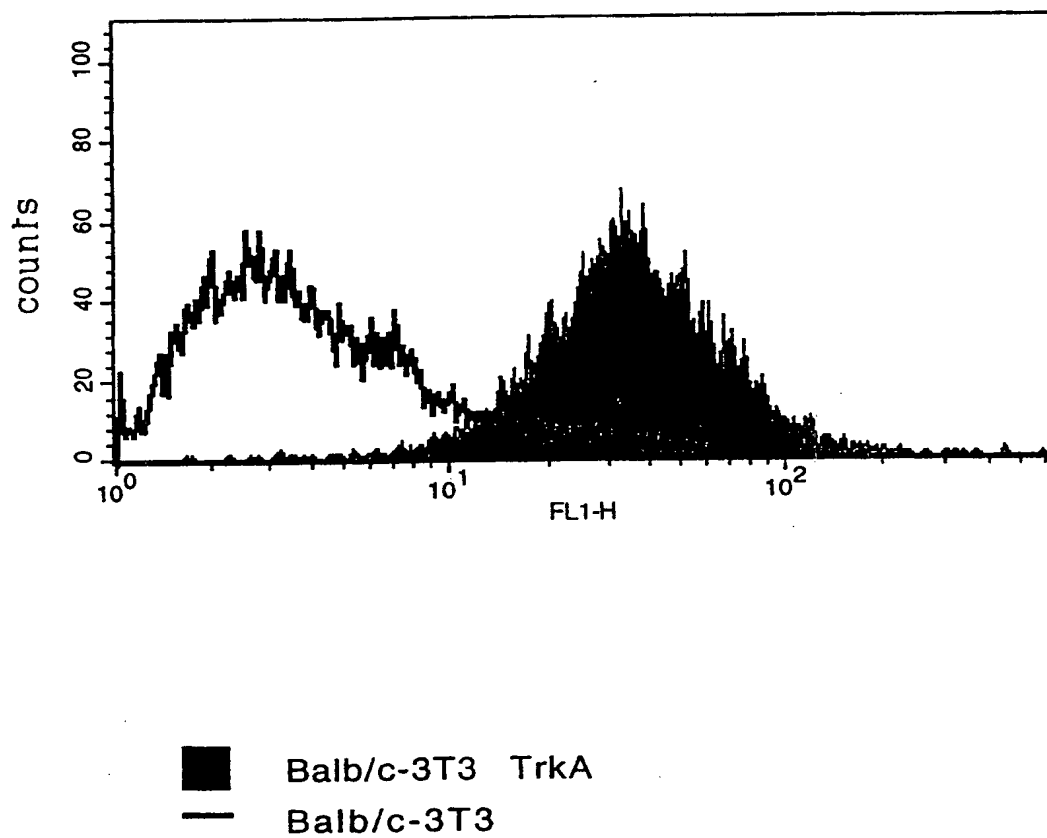


FIG. 3

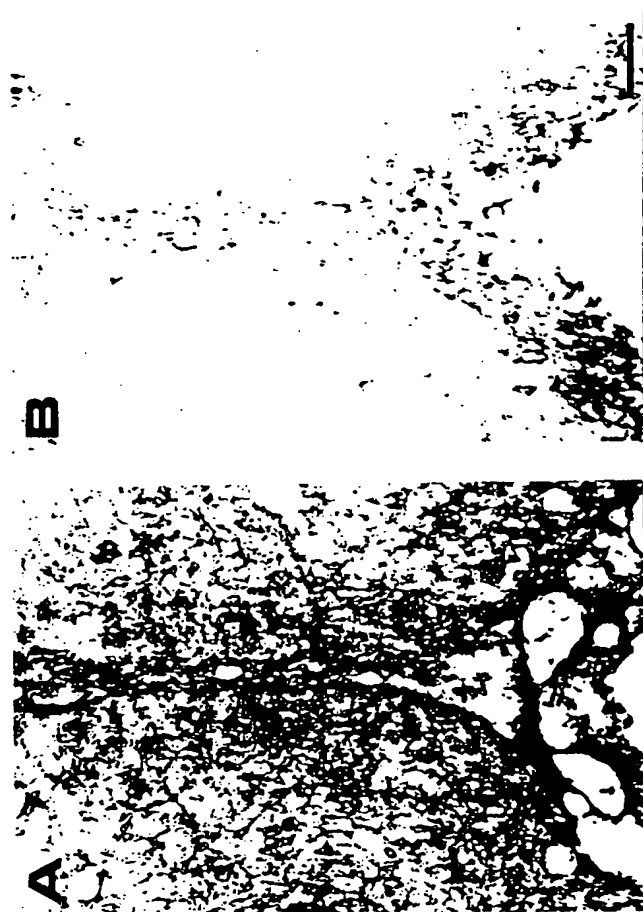


FIG. 4

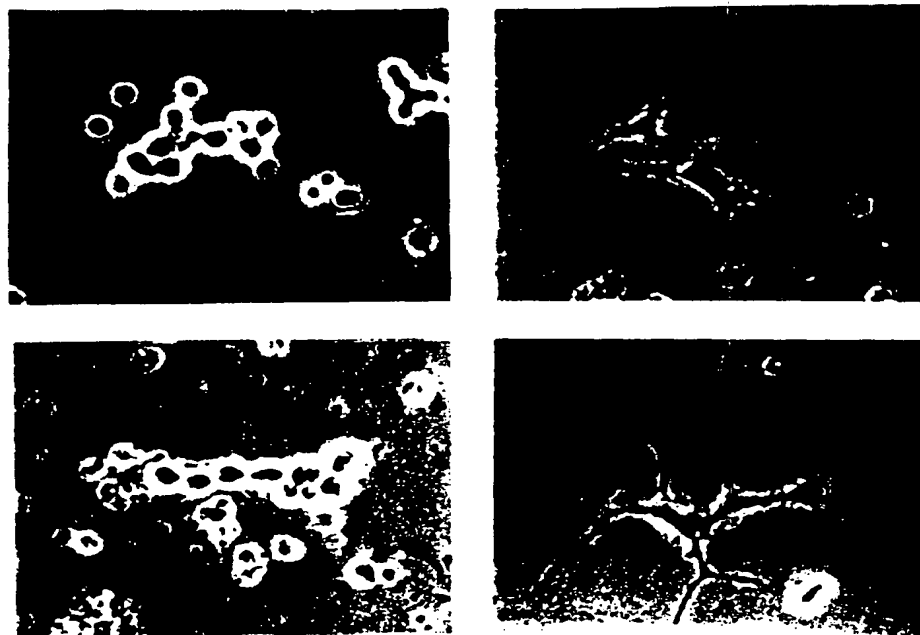


FIG. 5

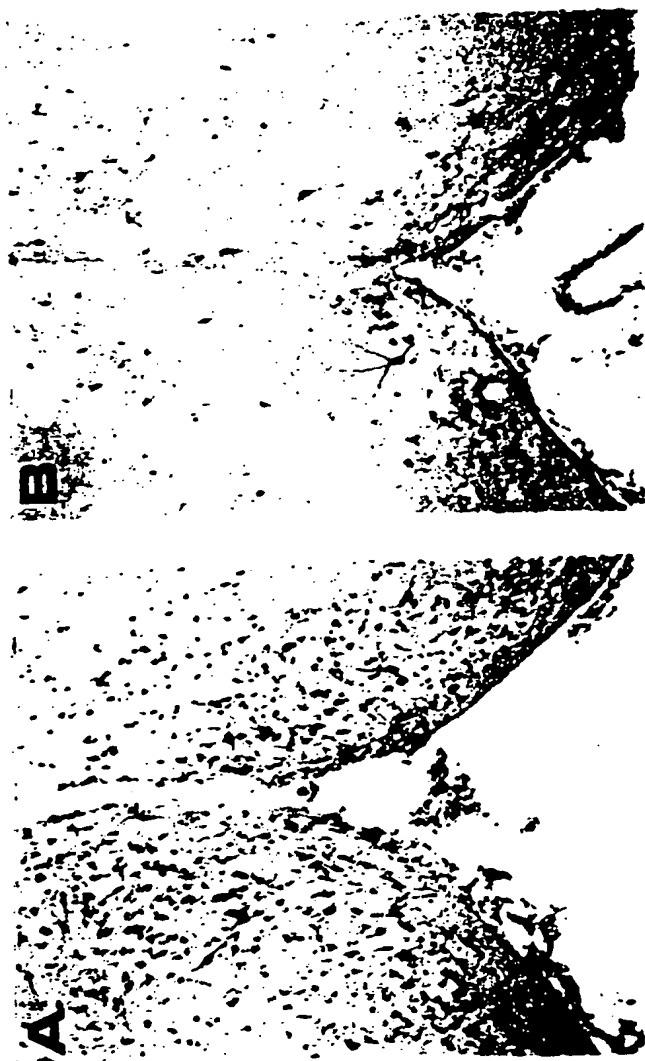


FIG. 6

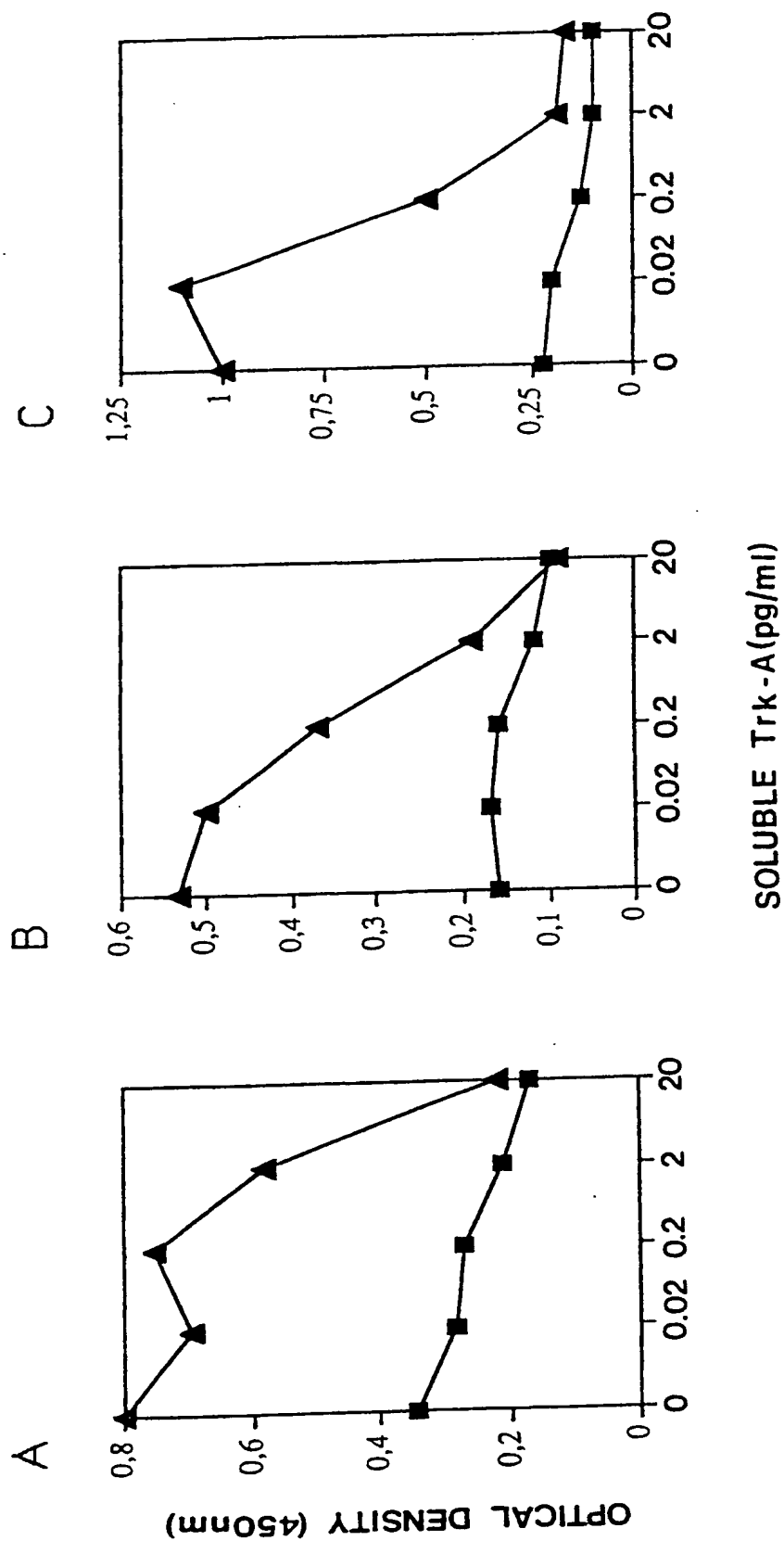


FIG. 7

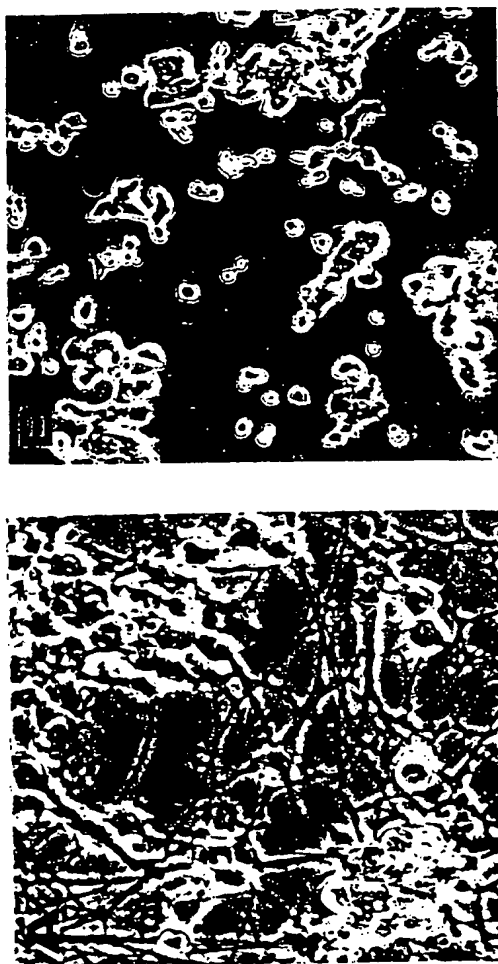


FIG. 8

SEQUENCE LISTING

<110> Societ... Italiana per la Ricerca Scientifica srl

<120> Anticorpi monoclonali ...

<130> Novach

<140>

<141>

<160> 2

<170> PatentIn Ver. 2.0

<210> 1

<211> 888

<212> DNA

<213> human ScFv antibody

<220>

<221> CDS

<222> (1)..(888)

```

<400> 1
atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc gca 48
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
      1              5              10              15

gca agc ggc gcg cat gcc gat att gtt ctc tcc cag tct cca gca atc 96
Ala Ser Gly Ala His Ala Asp Ile Val Leu Ser Gln Ser Pro Ala Ile
      20              25              30

atg tct gca tct cta ggg gag gag atc acc cta acc tgc agt gcc agc 144
Met Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser
      35              40              45

tcg agt gta agt tac atg cac tgg tac cag cag aag tca ggc act tct 192
Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser
      50              55              60

ccc aag ctc ttg att tat act aca tcc aac ctg gct tct gga gtc cct 240
Pro Lys Leu Leu Ile Tyr Thr Thr Ser Asn Leu Ala Ser Gly Val Pro
      65              70              75              80

tct cgc ttc agt ggc agt ggg tct ggg acc ttt tat tct ctc aca atc 288
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile
      85              90              95

agt agt gtg gag gct gaa gat gct gcc gat tat tac tgc cat cag tgg 336
Ser Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp
      100              105              110

agt agt tat cca tgg acg ttc ggt gga ggc acc aag ctg gaa atc aaa 384
Ser Ser Tyr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
      115              120              125

cgt tcc gga ggg tcg acc ata act tcg tat aat gta tac tat acg aag 432
Arg Ser Gly Gly Ser Thr Ile Thr Ser Tyr Asn Val Tyr Tyr Thr Lys
      130              135              140

tta tcc tcg agc ggt acc gag gtg aag ctg atg gag tct ggg gga ggt 480
Leu Ser Ser Ser Gly Thr Glu Val Lys Leu Met Glu Ser Gly Gly Gly

```

```
<210> 2
<211> 295
<212> PRT
<213> human ScFv antibody
```

```

<400> 2
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1           5           10           15
Ala Ser Gly Ala His Ala Asp Ile Val Leu Ser Gln Ser Pro Ala Ile
          20           25           30
Met Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser
          35           40           45
Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser
          50           55           60
Pro Lys Leu Leu Ile Tyr Thr Thr Ser Asn Leu Ala Ser Gly Val Pro
          65           70           75           80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile
          85           90           95

```

Ser Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp
 100 105 110
 Ser Ser Tyr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 115 120 125
 Arg Ser Gly Gly Ser Thr Ile Thr Ser Tyr Asn Val Tyr Tyr Thr Lys
 130 135 140
 Leu Ser Ser Ser Gly Thr Glu Val Lys Leu Met Glu Ser Gly Gly Gly
 145 150 155 160
 Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
 165 170 175
 Phe Thr Phe Ser Thr Tyr Thr Met Ser Trp Ala Arg Gln Thr Pro Glu
 180 185 190
 Lys Arg Leu Glu Trp Val Ala Tyr Ile Ser Lys Gly Gly Gly Ser Thr
 195 200 205
 Tyr Tyr Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
 210 215 220
 Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp
 225 230 235 240
 Thr Ala Leu Tyr Tyr Cys Ala Arg Gly Ala Met Tyr Gly Asn Asp Phe
 245 250 255
 Phe Tyr Pro Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
 260 265 270
 Ser Ala Ser Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser
 275 280 285
 Thr His His His His His His
 290 295

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number
WO 00/73344 A3

(51) International Patent Classification⁷: **C07K 16/28**,
C12N 15/13, 15/63, 5/16, A01K 67/00, A61K 39/395,
48/00, 38/17, G01N 33/577 // (A61K 39/395, 38:17)

(21) International Application Number: **PCT/IT00/00218**

(22) International Filing Date: **26 May 2000 (26.05.2000)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
RM99A000333 **26 May 1999 (26.05.1999)** **IT**

(71) Applicant (for all designated States except US): **SIRS
SOCIETA' ITALIANA PER LA RICERCA SCIEN-
TIFICA SRL [IT/IT]; Via Giovanni Paisiello, 47/c,
I-00198 Roma (IT).**

(72) Inventor; and

(75) Inventor/Applicant (for US only): **NOVAK, Michal, M.
[SK/SK]; Svabinskeho Str. n. 1, 851 01 Bratislava (SK).**

(74) Agents: **BANCHETTI, Marina et al.; Ing. Barzanò &
Zanardo Roma S.p.A., Via Piemonte, 26, I-00187 Roma
(IT).**

(81) Designated States (national): **AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK,
DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.**

(84) Designated States (regional): **ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).**

Published:

— *With international search report.*

(88) Date of publication of the international search report:
28 June 2001

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: **MONOCLONAL ANTIBODIES, SYNTHETIC AND BIOTECHNOLOGICAL DERIVATIVES THEREOF ACTING
AS NGF-ANTAGONIST MOLECULES**

(57) Abstract: **Monoclonal antibodies, synthetic and biotechnological derivatives thereof (ScFv or others) are able to recognise the
NGF high affinity receptor, TrkA, and act as NGF-antagonist molecules. Pharmacological compositions for therapy, gene therapy,
diagnostics of neurological pathologies are also described. Transgenic animal models to study such pathologies are also described.**

WO 00/73344 A3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 00/00218

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/28 C12N15/13 C12N15/63 C12N5/16 A01K67/00
A61K39/395 A61K48/00 A61K38/17 G01N33/577
/(A61K39/395, 38:17)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N A61K G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, CHEM ABS Data, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DEBEIR T ET AL: "A nerve growth factor mimetic TrkA antagonist causes withdrawal of cortical cholinergic boutons in the adult rat."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 MAR 30) 96 (7) 4067-72. , XP000941791</p> <p>the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-24

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

20 December 2000

Date of mailing of the international search report

05/01/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Mennessier, T

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/IT 00/00218

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>LESAUTEUR L ET AL: "POTENT HUMAN P140-TRKA AGONISTS DERIVED FROM AN ANTI-RECEPTOR MONOCLONAL ANTIBODY" JOURNAL OF NEUROSCIENCE,US,NEW YORK, NY, vol. 16, no. 4, 15 February 1996 (1996-02-15), pages 1308-1316, XP000645295 ISSN: 0270-6474 cited in the application the whole document</p>	1-24
P,X	<p>--- CATTANEO A ET AL: "Functional blockade of tyrosine kinase A in the rat basal forebrain by a novel antagonistic anti-receptor monoclonal antibody." JOURNAL OF NEUROSCIENCE, (1999 NOV 15) 19 (22) 9687-97. , XP000945020 the whole document</p> <p>-----</p>	1-24